

MICROSCOPY

In this General Microbiology class you will be using a Brightfield Compound Light Microscope. This light microscope is an important tool in the study of microorganisms. It provides the opportunity to witness microbes that cannot be seen with the naked eye.

The compound light microscope uses visible light to directly illuminate specimens in a two-lens system. These two lenses are the **ocular lens** in the eyepiece and the **objective lens** in the revolving nosepiece.

The compound light microscopes has the following components:

- **Illuminator:** the light source in the base of the microscope.
- **Abbe Condensor:** a two lens system that collects and concentrates light from the illuminator and directs it to the iris diaphragm.
- **Iris Diaphragm:** regulates the amount of light entering the lens system.
- **Mechanical Stage:** a platform used to place the slide on which has a hole in the center to let light from the illuminator pass through.
- **Stage clips** to hold the slide in place.
- **Body tube:** Houses the lens system that magnifies the specimens
Upper end of body tube -
- **Ocular pieces:** what you view through
Lower end of body tube -

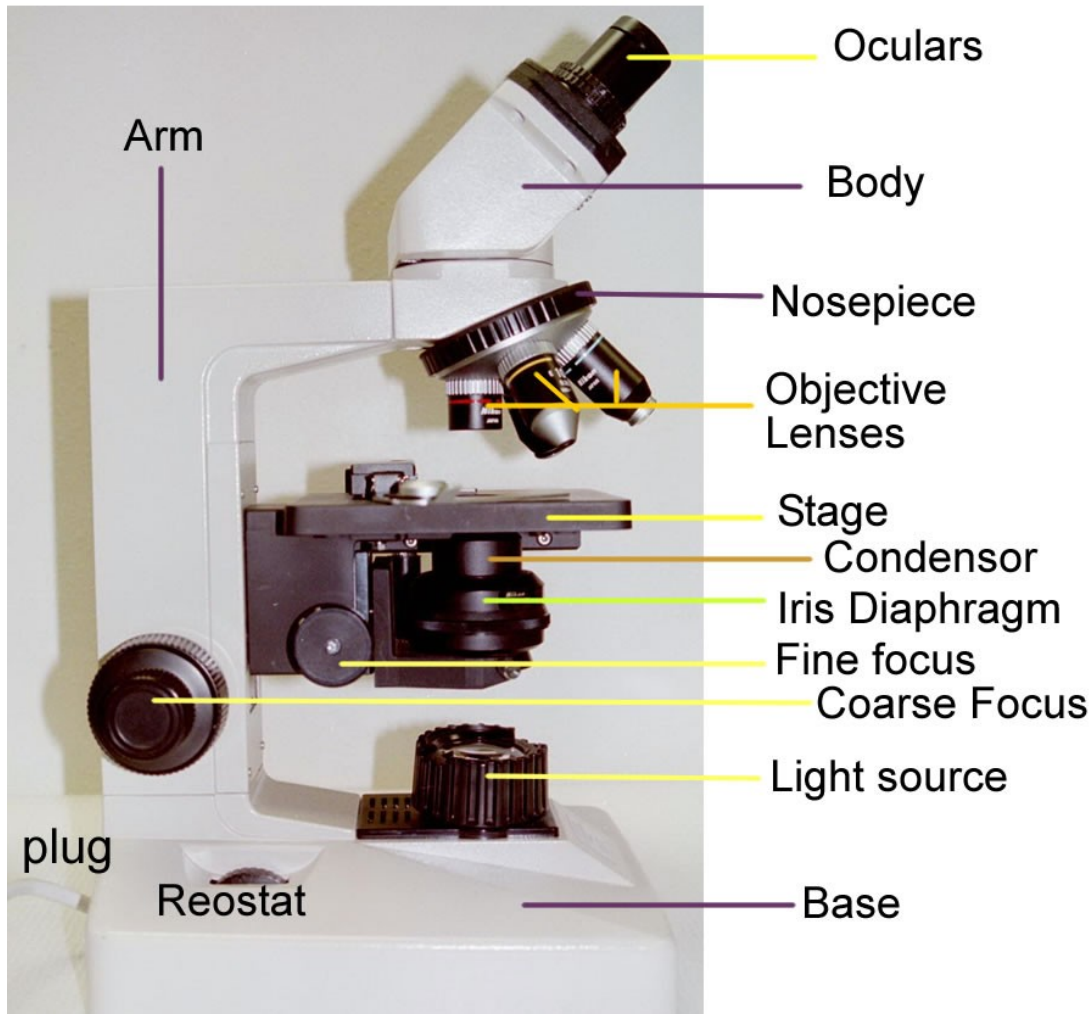
- **Nose-piece:** revolves and contains the objectives

The principles of microscopy are basically that a light microscope magnifies small objects and makes them visible. The science of microscopy is based on the following concepts and principles:

Magnification is simply the enlargement of the specimen. In a compound lens system, each lens sequentially enlarges or magnifies the specimen. The **objective lens** magnifies the specimen, producing a **real image** that is then magnified by the **ocular lens** resulting in the final image. The **total magnification** can be calculated by multiplying the objective lens value by the ocular lens value.

Light Microscope

Parts



Resolving power is the ability of a lens to show two adjacent objects as discrete entities. Resolving power is also dependent on the **refractive index** or the bending power of light. Because air has a lower refractive index than glass, light waves have a tendency to bend and scatter as they pass through the air from the glass slide to the objective lens.

Addition of **immersion oil**, which has the same refractive index as glass, diminishes the loss of refracted light and improves resolution.

Contrast is the ability to distinguish an object from its background. Since most microbes are relatively transparent when viewed under a standard light microscope they are difficult to identify. Using a stain (labs 2-5) that will bind to the microorganism and not the glass slide, dramatically enhances their contrast enabling them to be observed more clearly.

Depth-of-focus is the “thickness” of the sample that appears in focus at a particular magnification. As the magnification increases the depth-of-focus decreases, or the “slice” of the sample that appears in focus gets thinner. Many of the newer compound microscopes are **par focal**, which means that if one objective lens has the object in focus, and you go to the next objective lens, only minor adjustment (fine focus) is needed to bring the image back into focus. This is due to the fact that as you increase the magnification, and thus the slice of the sample that appears in focus becomes “thinner”, the correct plane-of-focus will always be within the depth of focus of the previous objective. After you get the sample into focus at scanning or low power using the course adjustment knob, you should only have to use the fine focus knob at the higher magnifications.

Field-of-View is the area of the slide that you are observing through the microscope. As you increase the magnification the actual area of the slide that you are looking at is getting smaller. You can think of the field-of-view as a dartboard. At low

magnification you are able to see the entire dartboard, but as you increase the magnification you are only observing the bulls-eye, a much smaller portion of the dartboard.

These microscopes are also **par central**, which refers to the ability to keep an object in the middle of your field-of-view when changing from one objective to another. It is useful to remember this as you are increasing magnification. Always keeping your sample in the center of your field-of-view will avoid unnecessary “searching” of the slide for your sample.

Working distance is the distance between the objective and the slide. As you increase magnification (by using more powerful objective lenses) the working distance decreases. So much so that by the time you are using the oil-immersion objective (100X) the objective is almost touching the slide, allowing the immersion oil to “connect” the slide and objective. It is important to consider working distance in a number of applications, but practically there are two reasons you should be aware of your working distance. The first is so that you do not inadvertently push the objective through the slide, causing damage to the objective and your sample slide. The second is to estimate whether you are in the correct plane-of-focus.

Care of Microscopes Microscopes are very expensive pieces of scientific equipment and must be treated with care. Each pair of students will be assigned a microscope to use throughout the semester, and will be required to sign a microscope agreement form

acknowledging responsibility for that microscope. Some basic rules of microscope care include the following:

(1) Always carry a microscope with two hands, one on the base and one on the arm. (2) Use the coarse focus knob on the lowest objective only. NEVER use coarse focus on

high power or oil immersion, or you may damage the objective lenses.

- . (3) Always clean all lenses thoroughly with lens paper and lens cleaner before putting away. Immersion oil which is not removed immediately can dry on the lenses, making it difficult to view any specimen. Dried immersion oil is also quite difficult to remove from the lenses.
- . (4) Always store the microscope with the lowest objective (4X) in place. NEVER store the microscope with the oil immersion objective in place, as it can damage the lens.

MATERIALS NEEDED:

- Commercially prepared slides of: Any *Staphylococcus* species
- Any *Bacillus* species
- *Saccharomyces cerevisiae* (yeast)
- Microscope
- Immersion Oil
- Lens Paper
- Lens Cleaner
- Microscope

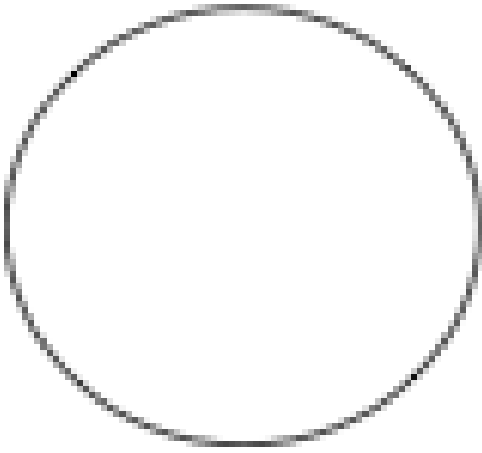
PROCEDURE:

1. Place the slide on the stage and use the coarse and fine focus knobs to bring the specimen into focus under 4X magnification. **Tip:** Bring the stage all the way to the highest position with the coarse focus knob. While looking through the ocular, gradually turn the knob to lower the stage until the specimen comes into focus. Once you have it in focus with the coarse focus knob, fine-tune with the fine focus.
2. Turn the revolving nose piece to bring the 10X objective into place and observe the specimen. Repeat with the 40X objective. Draw several cells observed under 40X in the spaces provided below.
3. Turn the revolving nose piece to bring the 100X objective into place and observe the specimen. Move the nosepiece slightly so you can add a drop of immersion oil to the slide, then bring the oil immersion objective back into place and fine-tune the focus. Observe the specimen. You may need to turn down the amount of light in order to observe the specimen under oil immersion.
4. Draw several cells observed under 100X in the spaces provided below.
5. Repeat steps 1-4 for the remaining prepared slides.
6. When finished, clean all lenses thoroughly with

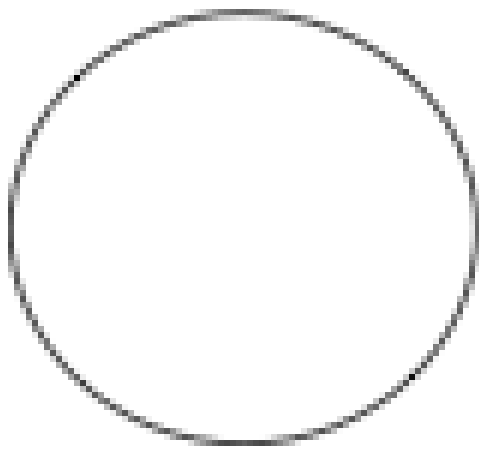
lens paper and lens cleaner. Have your instructor check your microscope to make sure it is clean. _____ (Instructor's initials)

7. Store the microscope according to the instructions written on the inside of your microscope cabinet.

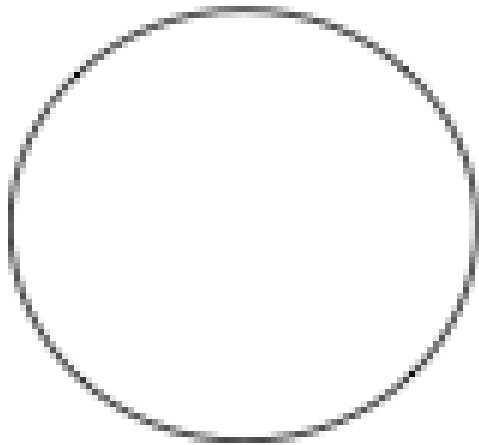
RESULTS:



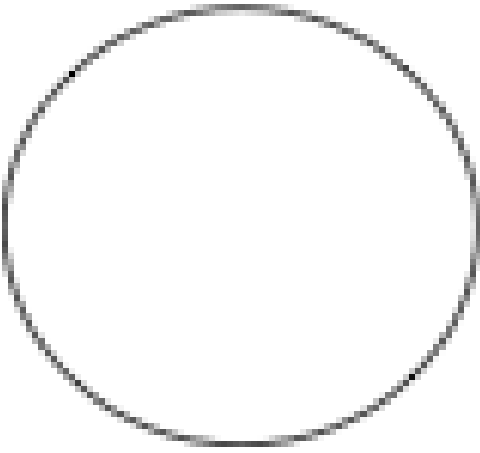
400X *Staphylococcus*



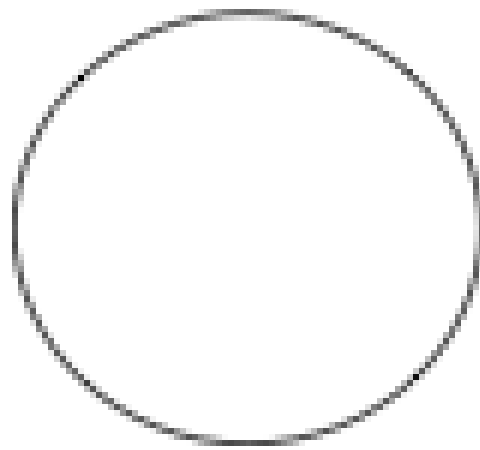
400X *Bacillus*



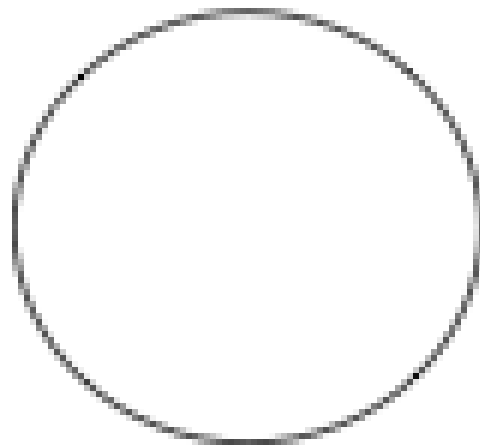
400X *Saccharomyces*



1000X *Staphylococcus*



1000X *Bacillus*



1000X *Saccharomyces*

STUDY QUESTIONS:

1. State the purpose of each of the following microscope components:
 - a. Condenser
 - b. Fine Focus
 - c. Coarse Focus
 - d. Iris
 - e. Diaphragm
 - f. Mechanical Stage Control
2. What is the purpose of adding immersion oil when using the 100X objective?
3. Compare the relative sizes of the bacteria (*Staphylococcus* and *Bacillus*) and yeast (*Saccharomyces*) under 10X, 40X, and 100X magnification.
4. Were you able to distinguish any internal structures, such as a nucleus, in any of the specimens viewed under oil immersion (100X)? Explain.
5. If the ocular lens has a magnification of 10X and the objective lens has a magnification of 40X, what is the total magnification?